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Chemical Carcinogenesis in the Tracheobronchial Epithelium

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Some of the recent work in pulmonary carcinogenesis is briefly reviewed. Morphologic studies of neoplastic and preneoplastic lesions of the human bronchi are compared with similar studies of carcinogenesis and epithelial regeneration in the hamster trachea. These studies suggest that bronchogenic carcinomas are typically complex mixtures of three basic phenotypes, the epidermoid and the mucous and dense-core granulated (endocrine) phenotypes. Pure forms of these phenotypes are rare, as different cells and even individual cells in single tumors express more than one phenotype. The clinical significance of such phenotypic variability is not yet known. Bronchial cell types which retain the capacity to divide include the mucous cell, the basal cell and perhaps the dense-core granulated cell. Studies of epithelial regeneration and preneoplastic lesions suggest that the mucous cell may be pivotal both in the response to injury and in carcinogenesis. Cigarette smoking is believed to be the major etiologic factor in bronchogenic carcinoma. Cigarette smoke contains initiators of carcinogenesis, but it contains a plethora of probable promoters and cocarcinogens as well. It is hypothesized that cigarette smoke may both initiate bronchial cells and promote carcinogenesis in cells which have previously been initiated by smoke or other factors. It is further hypothesized that the mucous cell is the major target for initiation and subsequent tumorigenesis. The ultimate phenotype(s) displayed by the tumor is suggested to result from the effect of microenvironmental factors upon the initiated cell and its progeny.

Introduction

The multiple phenotypes often encountered in descriptions and classifications of human and animal tracheobronchial neoplasms have long been baffling to many pathologists. On the one hand, it has been very difficult to separate these phenotypes into a rational classification, since there has been little or no evidence that morphologic characterizations are in any way meaningful from the standpoint of prevention, etiology, treatment or prognosis. But on the other hand, recent developments indicate that the tumor phenotype may have important etiologic, therapeutic, and prognostic significance. For example, small cell carcinomas appear to be relatively more frequent in uranium miners who smoke (*1*), adenocarcinomas appear to be increasing as a proportion of the whole (*2*) and small cell carcinomas with putative endocrine characteristics appear to be susceptible to some newer chemotherapeutic protocols (*3*).

Over the past several years, a variety of refined approaches have been developed with which the phenotypic characteristics of human and experimental animal tracheobronchial neoplasms can be characterized more effectively. These include improved methods of light microscopic cytochemistry, electron microscopy, immunoperoxidase techniques for a variety of hormonal and other markers including fetal antigens, and *in vitro* methods. At the same time, many developments have occurred in the area of animal models which can now be carefully compared with human neoplasms and the evolution of tracheobronchial neoplasia further characterized (*4,5*). Moreover, through the use of *in vitro* cultures of tracheobronchial epithelium from human and experimental animals, direct comparisons can now be made in terms of preneoplasia and neoplasia (*6,7*).

We will confine our comments to neoplasms arising in the tracheobronchial epithelium, excluding the bronchioles and alveoli. During the past several years we have studied and characterized in detail approximately 250 human bronchial neoplasms and have compared these in detail with animal models, specifically, the benzo(a)pyrene–ferric oxide model in hamsters, developed by Saffiotti and co-workers (*8*). We have also conducted studies *in vitro* in both human and animal tracheobronchial epithelium in order to describe the response to carcinogens and promoters (*9–17*) and have been engaged in studies of epithelial repair in the hamster trachea *in vivo* (*18–21*). Some of the results of these studies are briefly summarized in this review.

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Study of Human Bronchial Neoplasia

Methods have been developed in our laboratories which permit virtually any technique in cell or molecular biology, biochemistry and immunology to be applied to studies of human tissues, in this case the tracheobronchial epithelium. We have reviewed these methods elsewhere (*7*), and they are only briefly summarized here.

Obtainment, Transport, Storage and Culture

Human tissues are obtained at the time of surgery or immediate autopsy and transported in antibiotic-containing L-15 medium to the laboratory. Depending on temperature, tissues can be stored for various periods of time ranging up to several days at 0–4°C, or they can be frozen. In most cases, however, tissues are utilized within 24 hr of obtainment. A variety of methods has been utilized for explant culture of pieces up to 1 cm on a side for periods of up to 1 year. Conditions have been established which maximize differentiation of the epithelium as well as conditions which permit maximum cell division. Recently Lechner et al. (*22*) have developed methods for long-term cell culture. We, therefore, have a variety of techniques which can permit explant or cell culture and which allow evaluation of responses to injury including carcinogen treatment.

Xenograft Methods

Pieces or cylinders of bronchi can be grafted subcutaneously in the athymic or nude mouse (*23*). When small tubes of segmental, subsegmental, or smaller bronchi are utilized, the ends seal off and are covered by the dividing epithelium. Following an initial period of injury and organization, the graft becomes vascularized and the respiratory epithelium is maintained for long periods of time, up to 1 year. The secretory products, containing abundant mucosubstances, accumulate in the lumen of the graft. If flat pieces, i.e., 0.5 to 1 cm on a side, are grafted subcutaneously, the epithelial cells divide to line a cyst, the stroma of which is provided by the host. The epithelium lining the cyst becomes differentiated into a very normal-appearing, mucus-secreting pseudostratified columnar epithelium.

Utilizing such methods, one can study histogenesis of human tumors *in vitro* and in xenografts and compare results with animal tissues *in vitro*, in grafts or *in vivo*.

Animal Models of Pulmonary Neoplasia

Much work has been done on this subject during the past 10 years. Many new models *in vitro* and *in vivo* using various species and different routes of administration have been developed. These have been extensively reviewed by Nettesheim and Griesemer (*24*). Among the more important *in vivo* animal models are those involving inhalation, injection, transpleural insertion, and systemic administration of carcinogens. *In vitro* animal models have utilized both explant and cell cultures exposed to carcinogens. Recently, *in vivo*, *in vitro*, and *in vivo/in vitro* models have been developed (*25*). As mentioned elsewhere in this review, our laboratory has characterized a hamster model of tracheobronchial neoplasia (*8*) and compared it with lesions in human bronchi (*4,5,26,27*).

Tumor Phenotypes in Human Bronchial Neoplasia

A variety of cellular phenotypes, often mixed, is found in human bronchial neoplasms. This has resulted in various schemes for classification including the classification sponsored by the World Health Organization (*28*) and one developed in our laboratory (Table 1). Note that in both classifications many phenotypic mixtures are seen; in fact, in our experience in a surgical series, the most common phenotype involves mixtures of adeno- and epidermoid differentiation. In several papers and recent reviews, we have summarized the details of these classifications and their comparisons, which we do not attempt to repeat here (*16,27,29–32*).

The principal phenotypes that can be recognized are the epidermoid or "squamous carcinoma" phenotype; the adenocarcinoma phenotype possessing dense-cored granules which are presumed to represent an "endocrine" differentiation and the mucus-secreting adenocarcinoma phenotype. The adenocarcinoma phenotypes can appear either with or without gross glandular formation. Although the "pure" phenotypes are summarized in Table 2, it is to be emphasized that these very seldom if ever occur in actual tumors, as most if not all have various mixtures of the three basic types. By comparing the characteristics of the phenotypes in Table 2 with the classifications in Table 1, the mixtures of phenotypes can be deduced.

Preneoplastic Lesions and Histogenesis

The histogenesis of bronchial neoplasia in the human still remains uncertain though in recent years detailed studies of human neoplastic and preneoplastic lesions, as well as parallel detailed studies of animal models, have yielded much new information on their probable mode of origin. In this section we will review some current concepts in this area with a particular view towards understanding so-called preneoplastic lesions. By preneoplastic lesions we are referring to lesions that occur prior to the development of invasive cancer in both man and experimental models. Such lesions in the bronchus include epidermoid (squamous) metaplasia with and without atypia and carcinoma *in situ*. These are considered to represent altered cell populations en

Table 1. Comparison of phenotypic and WHO (1981) tumor typing.

Phenotypic	WHO
Tumors without dense-cored granules[a]	
Epidermoid carcinoma (EC)	
Well–moderately differentiated	1. Squamous cell carcinoma
Poorly differentiated	5. Adenosquamous carcinoma[b]
Small cells	2. Small cell carcinoma
Large cells	4. Large cell carcinoma
Combined epidermoid and adenocarcinoma (CEAC)	
A. Epidermoid component well differentiated, Adeno component well differentiated	7b. Mucoepidermoid carcinoma
B. Epidermoid component, well differentiated, Adeno component poorly differentiated	1. Squamous cell carcinoma 5. Adenosquamous carcinoma[b]
C. Epidermoid component poorly differentiated, Adeno component well differentiated	
I. Tubular glands present, with or without mucus	3a. Acinar adenocarcinoma
II. Tubular glands minimal, with mucus	3d. Solid carcinoma with mucus
D. Epidermoid component, poorly differentiated, Adeno component, poorly differentiated	
Small cells	2. Small cell carcinoma
Large cells	4. Large cell carcinoma
Adenocarcinoma (AC)	
Well–moderately differentiated	
I. Tubular glands present, with or without mucus	3a. Acinar adenocarcinoma
II. Tubular glands minimal, with mucus	3d. Solid carcinoma with mucus
Poorly differentiated	
Tubular glands and mucus minimal	
Small cells	2. Small cell carcinoma
Large cells	4. Large cell carcinoma
Tumors with dense-cored granules[a]	
Carcinoid tumor[c]	6. Carcinoid tumor
Small cell carcinoma[d]	2. Small cell carcinoma
Atypical endocrine carcinoma[d]	1. Squamous cell carcinoma 3d. Solid carcinoma with mucus 4. Large cell carcinoma

[a] Tumors with dense-cored granules stain positive for neuron-specific enolase; tumors without the granules do not stain for this enzyme.

[b] Ducts lined by nonneoplastic Type II alveolar cells may be trapped in epidermoid cell nests giving the erroneous impression of adenosquamous carcinoma.

[c] Some carcinoid tumors are mucus-secreting.

[d] Small cell carcinomas and atypical endocrine tumors may exhibit an epidermoid phenotype. Some tumors also secrete mucus. Small cell tumors with epidermization and/or mucus secretion are called combined oat-cell carcinomas in the WHO classification.

Table 2. Lung tumor phenotypes: some phenotypic characteristics of "pure" bronchogenic carcinomas.[a]

	Keratin[b]	Lumens	Mucous granules	ER	Mitochondria	Golgi	Desmosomes	Dense-core granules
Epidermoid carcinoma	+ + +	–	–	±	Few	±	+ +	–
Adenocarcinomas								
Mucosubstance type	+/±	+	+	+ +	Numerous	+ +	+	–
Dense-core granule type	+/±	–	–	+	Variable	+	+	+

[a] This table is meant to indicate the "pure" phenotypes of human bronchial neoplasia. It is to be emphasized that these neoplasms do not typically occur in pure form, but instead usually exist as phenotypic mixtures.

[b] Keratohyaline in well-differentiated tumors.

route to malignant transformation. Most if not all of these lesions appear to be reversible in both man and experimental models. Currently carcinogenesis is conceived as occurring in at least two and possibly multiple stages. These are often termed initiation and promotion (*33*). Initiation appears to involve genetic events probably involving mutagenesis, for example, formation of carcinogen-DNA adducts, and it is considered to be irreversible. Promotion, on the other hand, is reversible, involves cell proliferation, ion shifts, e.g., Ca and/or Na influx, induction of marker enzymes such as ornithine decarboxylase (ODC), and possibly increased keratin

formation in the cytoplasm (*33*). Many of the preneoplastic lesions in human and animal tracheobronchial epithelium probably represent the action of promoters. Many compounds, in addition to initiators, are found in tobacco smoke and other effluents of combustion in industrial environments. Some such putative promoters in tobacco smoke include catechols, particulates, and formaldehyde (*1*). If such promoters are applied to human or animal tracheobronchial epithelium, lesions closely resembling known preneoplastic lesions are observed.

The Normal Tracheobronchial Epithelium

Tracheobronchial epithelia of humans and hamsters appear to share many similarities (*34,35*). However, certain differences, which may or may not be important, appear to exist between the tracheobronchial epithelia of rats and mice and humans.

It is important to recognize that the epithelium is truly pseudostratified in the sense that all cells seem to rest upon the basal lamina, but not all cells reach to the lumen. Cell types found in the tracheobronchial epithelium in man and animals are mucous cells, ciliated cells, basal cells and endocrine cells.

Mucous cells are characterized by having variable numbers of granules containing neutral and/or acidic mucosubstances, usually in the cell apices. These vary in number, from the numerous large granules which distend the cell in the so-called goblet cells, to the small inconspicuous granules found in what we have termed small mucous granule cells, depending on the state of the epithelium. Mucous cells rest on the basal lamina and extend to the lumen. They have well-developed rough endoplasmic reticulum and Golgi components, numerous mitochondria and an electron dense cytosol which distinguishes them even when only small portions of the cells can be seen. The mucous cells of hamsters seem to be similar to those in the human though the granules are often somewhat more electron dense in usual preparations. In the rat, cells which secrete mucosubstances may be somewhat different both in their electron microscopic (EM) and cytochemical characteristics (*36,37*). Mucous cells are capable of incorporating thymidine and undergoing division; they may be able to reconstitute the entire epithelium although specific cloning experiments are needed to test that hypothesis. They clearly seem to be of great importance in tracheal wound healing (*18–21*).

Ciliated cells, in addition to having cilia, differ from mucous cells in many ways. One of the useful characteristics in their identification at the EM level, is the electron lucency of their cytosol. This may imply modification of ion and water content as well as a decreased concentration of ribosomes and rough endoplasmic reticulum (ER). Ciliated cells do not incorporate thymidine and certainly cannot divide without modification. Studies on ciliary amputation followed by division have been performed in lower forms including protozoa; the application of this principle to mammalian respiratory ciliated cells has not been accomplished. In the human, abnormalities of cilia commonly exist even in putatively healthy individuals. Such abnormalities include multiple axonemes within a single cytoplasmic process, changes in the 9 + 2 arrangement (*38*) and alterations in basal bodies. Ciliated cells also seemingly have the property of easily dissociating from their neighbors following injury to the epithelium and being expectorated. We have recently found that treatment of explanted hamster tracheobronchial epithelium with amphotericin (*39*) results in abnormal cilia, possibly because of increased cell Na and diminished Na–Ca exchange and, therefore, increased cell Ca leading to a modified cytoskeleton.

Basal cells are located along the basal lamina and do not reach the lumen. They resemble mucous cells in the electron density of the cytosol but in addition have hemidesmosomes which attach them to the basal lamina. They often contain abundant keratin filaments. Basal cells incorporate thymidine and divide.

Scattered along the epithelium, sometimes reaching the lumen and sometimes not, are cells which have "dense-cored" granules. Presently it is not known whether or not these cells are purely endocrine. The frequency of these cells seems to differ not only during growth and development but also between anatomic sites within the airway and between species. In the normal epithelium these cells divide infrequently and apparently only in the fetal state. The granules are argyrophilic and can be demonstrated with the PAS-lead hematoxylin technique of Sorokin and Hoyt (*40*). At one time it was thought that these cells derived from migration of neural crest cells during embryonic development. This hypothesis has not been confirmed, and many investigators presume today that they must be derivatives of the original endodermal pouch which ultimately forms the tracheobronchial epithelium.

Embryology of the Tracheobronchial Epithelium

It is important to understand normal development of the tracheobronchial epithelium, because many features of development are recapitulated during epithelial regeneration, preneoplasia, and neoplasia. Although much remains to be learned about the details of embryogenesis, there is general agreement in the following. The early endodermal tubes which form the lung are lined by a single layer of unspecialized columnar cells containing abundant glycogen. The epithelium later becomes pseudostratified as ciliated, mucous, endocrine, and basal cells develop. As this occurs, glycogen is depleted. In the rabbit, primordial endocrine cells were the first specialized cells to be recognized (*41*).

Regeneration of the Tracheobronchial Epithelium

In order to understand the cellular events that occur during the development of neoplastic lesions, it is useful to examine those that occur during wound repair. There have been a number of studies over the years and we have recently reinvestigated and quantified this phenomenon (*18–21*). Our model is the hamster trachea, which exhibits morphologic similarity to the human bronchial epithelium and which has been used in carcinogenesis models reproducing rather faithfully the portfolio of phenotypes seen in the human. The injury that has been studied consists of simple mechanical removal of a linear strip of epithelium using a blunt probe. Using this technique the epithelium is entirely removed down to the basal lamina at a focal point. The events are characterized *ad seriatum* for periods of up to 1 week. Colchicine blockade and tritiated thymidine injections provide insight into the cellular kinetics involved in the phenomenon.

Following the initial wound, an acute inflammatory response occurs with a fibrinopurulent exudate. Within the first few hours the cells at the margins of the wound undergo a remarkable shape change from columnar to squamous and soon begin migrating to cover the basal lamina. This migration seems to involve mostly secretory (mucous) cells, though occasional basal cells and flattened ciliated cells with partially resorbed cilia can be observed. It also appears that at this time many ciliated cells are lost from the epithelium and are shed into the lumen. Bundles of filaments (probably actin) can be observed along the basilar aspects of the migrating cells. It should be noted that these initial events precede any cell division. By 24 hr the wound is covered and numerous mitoses are arrested in metaphase after colchicine blockade. Thymidine incorporation is noted in both basal cells and secretory cells but most mitoses occur in secretory cells. By 48 hr there is piling up of the cells in the region of the wound, producing typical epidermoid (squamous) metaplasia. The cells contain abundant keratin filaments and the epithelium is multilayered. Although the cells appear typically epidermoid in that they contain keratin, they also contain granules filled with mucosubstances. Because of this and because of the significantly greater number of mitoses in secretory cells than basal cells, it is inferred that these metaplastic cells are in large part derivatives of secretory cells. Some contribution from basal cells, however, cannot be totally excluded.

Some of the most superficial epidermoid metaplastic cells slough from the epithelium and by 72 hr reconstruction of the mucociliary epithelium is underway by the genesis of presecretory and preciliated cells, from division of mature secretory cells. Presecretory cells mature into mucus secreting cells, whereas preciliated cells show evidence of ciliogenesis at the cell apex in the form of fibrogranular areas and later as basal bodies, from which cilia develop. Over a period of 1 week, the normal pseudostratified mucociliary epithelium is restored (*18–21*).

It is apparent from these studies that epithelial wound healing in the hamster tracheal epithelium recapitulates some features of embryonic development but also resembles features of preneoplastic lesions, e.g., epidermoid metaplasia. It is not presently known whether wounding *per se* constitutes a stimulus for tumor promotion in the respiratory epithelium.

Preneoplastic Lesions

Several lines of evidence indicate that human bronchogenic neoplasms develop following the appearance of a series of preneoplastic cell populations. Evidence includes case control studies on defined human populations, such as smoking uranium miners who were followed by sputum cytology. In many cases, the progressive cytologic change was followed for over 10 years prior to the development of invasive bronchogenic carcinoma (*42*). These studies clearly show that a definite progression occurs from epidermoid metaplasia without atypia through epidermoid metaplasia with atypia, thereafter developing carcinoma *in situ*, and invasive cancer. It is evident that at any one phase of the disease, all of the previous stages exist, but a clear progression of the most advanced lesions can be followed. The sequence of change in the bronchus is thus rather similar to the progression of carcinomatous change that occurs in the human uterine cervix. It is significant that a similar, if not identical, sequence of events occurs in the benzo(a)pyrene ferric oxide model of bronchogenic carcinogenesis in the hamster (*43*).

On examining the so-called preneoplastic lesions in greater detail, one of the earliest expressions of chronic injury to the tracheobronchial epithelium is the appearance of numerous mucous cells, both goblet cells and small mucous granule cells. In typical lesions, large areas are free of ciliated cells. Since mucous cells can divide, this lesion is best interpreted as a mucous cell hyperplasia. Ciliated cells, as mentioned above, are very susceptible to injury and frequently detach from their neighbors, even while still alive and pass into the airway lumen. In some areas of mucous cell hyperplasia, more than one layer of cells may be seen (*26*). Based on animal studies and cytopathologic studies in humans, the next lesions to appear, presumably a result of more severe or longer standing injury, are areas of epidermoid (squamous) metaplasia. These are frequently scattered within the bronchi, and often abrupt transitions between such areas and areas of mucous cell hyperplasia are noted. In areas of epidermoid metaplasia, multiple layers of cells containing variable amounts of keratin are seen. Using stains for mucosubstances such as the PAS-Alcian blue technique, many of the keratinizing cells also contain mucosubstances (*26,32*). This occurs not only in cells at the surface but in cells several layers

down from the surface, thus approaching the basal lamina. These small granules of mucosubstance can be confirmed by EM cytochemistry. Already at this stage, the cells begin to acquire new surface markers; for example, carcinoembryonic antigen can be demonstrated in areas of epidermoid metaplasia (*16*).

Tumor Promotion in the Tracheobronchial Epithelium

The concept that carcinogenesis occurs in a stagewise process has been suspected since the work of Rous and Kidd (*44*), Berenblum (*45*), Mottram (*46*), and Boutwell (*47*). All these investigators observed the effectiveness of some hyperplasiogenic agents in promoting tumor formation following subcarcinogenic exposure of skin to carcinogens, especially polycyclic aromatic hydrocarbons. Berenblum (*45*) introduced the use of croton oil which contained very active tumor promoting agents, and later Hecker (*48*) and van Duuren (*49*) identified phorbol esters as the active components. Much of the literature has been reviewed by Slaga et al. (*50*).

The multistage concept of carcinogenesis postulates that the function of the initiator is to convert normal cells into latent tumor cells, possibly through unexpressed mutagenic effects. Such latent neoplastic cells persist for long periods of time—up to the lifetime of the animal, unless exposed to a promoter. Subsequent application of promoters results in hyperplasias and many benign neoplasms which, in the case of mouse skin, may regress, be retained, or progress to carcinomas. More carcinomas result as the dose of the initiators is increased with a corresponding decrease in the dose of promoter.

It should be emphasized that most knowledge and theory concerning promotion are based on the mouse skin system. Although this has been recently extended to other organ systems in experimental animals, including the liver and colon, experimental data are lacking on the role of promoters in any human organ system; the main clues so far come from epidemiologic studies.

As pointed out by Potter (*51*), the concept of promotion may be very important to our current concept of lung cancer as a result of cigarette smoking. In human populations, many people may be exposed to subcarcinogenic levels of a carcinogen and never develop cancer in their lifetime, even though they may have a substantial number of initiated cells. This possibility appeared in an editorial in Lancet (*52*). In this regard, Roe et al. (*53*) carried out experiments demonstrating promoter activity in cigarette smoke condensates and suggested that the relationship between smoking and lung neoplasia might be determined principally by its tumor promoting activity. Potter (*51*) concluded that direct support for this conclusion comes from the studies of Auerbach et al. (*54*), who made detailed studies on the bronchi of 216 carefully matched smokers, former smokers, and nonsmokers who had died of causes other than lung cancer. They found that 93.2% of the 3156 sections from 72 smokers contained atypical cells, while only 6% of 3436 sections from 72 "former smokers" contained such cells and only 1.2% of the sections from nonsmokers showed atypical cells. Hammond (*55*) expressed a theory of carcinogenesis which is compatible with current concepts of initiation and promotion, in that it involves both somatic mutation (initiation) and hyperplasia, metaplasia, and atypia of the bronchial epithelium in the smoke environment (promotion). Hammond went on to infer that if an individual ceases smoking, the more normal microenvironment results in reversal of the proliferated, sometimes atypical cells (*55*).

In the skin, the most potent promoters are wounding (*56*) and the fatty acid esters of phorbol. These esters have strict structure-function relationships, their potency in part determined by the length of fatty acid ester groups, an optimum being about 14 carbons. The most potent of the phorbol esters so far characterized is 12-0-tetradecanoylphorbol-13-acetate (TPA). This compound is active both *in vivo* and *in vitro* in minute doses, following which there are multiple biochemical and morphologic changes. These include stimulation of macromolecular synthesis leading to hyperplasia, stimulation of polyamine synthesis, prostaglandin synthesis, protease production, alterations of cell membrane enzymes, phospholipids and glycoproteins, induction of sister chromatid exchange, altered differentiation, and altered responses to other growth controlling factors. Many of these effects have been demonstrated in skin and other cells in both experimental animals and man. So far, the critical responses for tumor promotion have not been determined; however, the pleiotropic effects induced by these agents at nanomolar concentrations and their discrete structure-activity relationships suggest a hormone-like action.

There is a great need for expansion of knowledge concerning the effects of cocarcinogens and promoters in the tracheobronchial epithelium (*25*). A number of cocarcinogens and promoters have been suspected or identified in both human and experimental bronchial neoplasia. These include several metals such as chromium, nickel and arsenic; particulates such as asbestos and ferric oxide; TPA; butylated hydroxytoluene (BHT) in mouse pulmonary adenomas; and a long list of compounds found in the gaseous and particulate phases of tobacco smoke. In tobacco smoke there is a wide variety of cocarcinogens including pyrene, fluoranthene, benzo[g,h,i]perylene, benzo(e)pyrene, naphthalenes, 1-methylindoles, 9-methylcarbazoles, 4-4'-dichlorostillbene and catechol (*1*), and a variety of poorly characterized acidic components in the gas phase which include formaldehyde, acrolein, acetaldehyde, and possibly the ciliotoxic agents hydrogen cyanide and carbon monoxide. It is entirely conceivable that the principal carcinogenic effect of cigarette smoking is the continuous exposure of the bronchial epithelium to a wide variety of promoters and cocarcinogens.

Recently, much attention has been directed in various laboratories including our own to the possible impor-

tance of rapid membrane and cytoskeletal alterations in tumor promotion. Such effects include rapid influx of Na followed by Ca, formation of superoxide radicals which further modify the cell membrane, modulation of cyclic AMP and cyclic GMP, modification of the cytoskeleton, and alteration of cell pH. We have recently summarized evidence that modification of Ca, possibly resulting from diminished Na-Ca exchange, could result in a number of the consequences including microtubule dissolution with possible subsequent cell division, increased expression of keratin filaments, activation of protein kinases, and modification of cell-cell interactions specifically diminution of gap junctions (*57*). Such modifications of cell ions might also be directly or indirectly related to the increased numbers of "dark" cells which occur in both skin and tracheobronchial epithelium following TPA application (*58,59*).

Comments

It should be apparent from the foregoing that the phenotypic characteristics of human and animal tracheobronchial neoplasms are complex. This apparent complexity may be important in that it reflects the histogenesis and possibly the etiologic risk factors and prognosis.

A variety of recent studies indicate that the cellular microenvironment can affect, if not determine, the phenotypic characteristics of cells. For example, vitamin A deficiency is associated with epidermoid metaplasia, while vitamin A excess results in excessive production of mucus. Anoxia and ischemia (common in neoplasms) are associated with increased intracellular calcium (*57*). Growth of human bronchial epithelium in high Ca medium is associated with increased cytoplasmic keratin (*22*) and treatment of hamster trachea *in vitro* with the Ca ionophore A23187 is associated with epidermoid metaplasia (*17*). Such considerations lead us to believe that the ultimate phenotype of human bronchial neoplasms is influenced largely by the microenvironment. Whether or not these tumor phenotypes exert any important influence on the biology of human bronchial neoplasia remains to be determined. In the same context, it has been reported that the phenotype of a bronchial neoplasm can change as a function of time in a given patient. Furthermore, the phenotype of the primary tumor may differ from that of the distant metastases.

In any case, our studies lead us to believe that the mucous cell of the tracheobronchial epithelium plays an important role in wound repair, preneoplasia, and neoplasia, which suggests that a high priority in the future should be given to studies which are designed to characterize the normal and abnormal biology of this pivotal cell type. Furthermore, it appears from our studies that although tumors can at least in theory arise from any or all of the cells that are capable of division, all of the phenotypes described in Table 2 can develop, perhaps dependent on the environment and/or the risk factors and carcinogens involved.

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REFERENCES

1. Harris, C. C. Respiratory carcinogenesis and cancer epidemiology. In: Lung Cancer: Clinical Diagnosis and Treatment (M. J. Straus, Ed.), Grune and Stratton, New York, 1983, pp. 1–20.
2. Vincent, R. G., Pikren, J. W., Lane, W. W., Bross, I., Takita, H., Houten, L., Gutierrez, A. C., and Rzepka, T. The changing histopathology of lung cancer. A review of 1682 cases. Cancer 39: 1647–1655 (1977).
3. Straus, M. J., Selawry, O. S., and Wallach, R. A. Chemotherapy in lung cancer. In: Lung Cancer: Clinical Diagnosis and Treatment, 2nd ed. (M. J. Straus, Ed.), Grune and Stratton, New York, 1983, pp. 261–283.
4. Becci, P. J., McDowell, E. M., and Trump, B. F. The respiratory epithelium. IV. Histogenesis of epidermoid metaplasia and carcinoma in situ in the hamster. J. Natl. Cancer Inst. 61: 577–586 (1978).
5. Becci, P. J., McDowell, E. M. and Trump, B. F. The respiratory epithelium. VI. Histogenesis of lung tumors induced by benzo[a]pyrene-ferric oxide in the hamster. J. Natl. Cancer Inst. 61: 607–618 (1978).
6. Saffiotti, U., and Harris, C. C. Carcinogenesis studies on organ cultures of animal and human respiratory tissues. In: Carcinogens: Identification and Mechanisms of Action (M. D. Anderson Hospital and Tumor Institute Monograph) (A. C. Griffin, and C. R. Shaw, Eds.), Raven Press, New York, 1979, pp. 67–84.
7. Trump, B. F., and Harris, C. C. Human tissue in biomedical research (editorial). Hum. Pathol. 10: 245–248 (1979).
8. Saffiotti, U., Cefis, E., and Kolb, L. A method for the experimental induction of bronchogenic carcinoma. Cancer Res. 28: 104–124 (1968).
9. Harris, C. C., Trump, B. F., Grafstrom, R., and Autrup, H. Differences in metabolism of chemical carcinogens in cultured human epithelial tissues and cells. In: Mechanisms of Chemical Carcinogenesis (C. Harris, and P. Cerutti, Eds.), A. L. Liss, New York, 1982, pp. 289–298.
10. Harris, C. C., Trump, B. F., Autrup, H., Hsu, I. C., Haugen, A., and Lechner, J. Studies of host factors in carcinogenesis using cultured human tissues and cells. In: Host Factors in Human Carcinogenesis (J. Bartsch and A. Armstrong, Eds.), International Agency for Research on Cancer (IARC Scientific Publications No. 39), Lyon, 1982, pp. 497–514.
11. Autrup, H., Grafstrom, R., and Harris, C. C. Metabolism of chemical carcinogens by tracheobronchial tissues. In: Organ and Species Specificity in Chemical Carcinogenesis (J. Rice, R. Langenbach, and S. Nesnow, Eds.), Plenum Press, New York, 1982, pp. 473–495.
12. Haugen, A., and Harris, C. C. Asbestos carcinogenesis: Asbestos interactions and epithelial lesions in cultures of human tracheobronchial tissues and cells. Recent Adv. Cancer Res. 82: 32–42 (1982).
13. Harris, C. C., and Autrup, H. Interspecies, interindividual and intertissue variations in benzo[a]pyrene metabolism. In: Luftverunreiningung durch Polycyclische Aromatische Kohlenwasserstoffe-Erfassung und Bewertung, Dusseldorff, VDI-Bericht Nr. 358, 1980, pp. 293–300.
14. Stoner, G. D., Harris, C. C., Myers, G. A., Trump, B. F., and Connor, R. D. Putrescine stimulates growth of human bronchial epithelial cells in primary culture. In Vitro 16: 399–406 (1980).
15. Harris, C., Autrup, H., Connor, R., Barrett, L., McDowell, E., and Trump, B. Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 194: 1067–1969 (1976).
16. Trump, B. F., Wilson, T., and Harris, C. C. Recent progress in the pathology of lung neoplasms. In: Lung Cancer (S. Ishikawa, Y. Hayata, and K. Suemasu, Eds.), Excerpta Medica, Amsterdam, 1982, pp. 101–124.
17. Saladino, A. J., Berezesky, I., Resau, J., and Trump, B. F.

Ionophore A23178 causes hyperplasia in hamster tracheal epithelium. Fed. Proc. 41: 740 (1982).
18. McDowell, E. M., Becci, P. J., Schürch, W., and Trump, B. F. The respiratory epithelium. VII. Epidermoid metaplasia of hamster tracheal epithelium during regeneration following mechanical injury. J. Natl. Cancer Inst. 62: 995–1008 (1979).
19. Keenan, K. P., Combs, J. W., and McDowell, E. M. Regeneration of hamster tracheal epithelium after mechanical injury. I. Focal lesions, quantitative morphologic study of cell proliferation. Virchows Arch. Pathol. 41: 193–214 (1982).
20. Keenan, K. P., Combs, J. W., and McDowell, E. M. Regeneration of hamster tracheal epithelium after mechanical injury. II. Multifocal lesions: Stathmokinetic and autoradiographic studies of cell proliferation. Virchows Arch. Pathol. 41: 215–229 (1982).
21. Keenan, K. P., Combs, J. W., and McDowell, E. M. Regeneration of hamster tracheal epithelium after mechanical injury. III. Large and small lesions. Comparative stathmokinetic and single pulse and continuous thymidine labeling autoradiographic studies. Virchows Arch. Pathol. 41: 231–252 (1982).
22. Lechner, J. F., Haugen, A., Autrup, H., McClendon, I. A., Trump, B. F., and Harris, C. C. Growth of epithelial cells from normal adult human bronchus. Cancer Res. 41: 2294–2306 (1981).
23. Valerio, M. G., Fineman, E. L., Bowman, R. L., Harris, C. C., Stoner, G. D., Autrup, H., Trump, B. F., McDowell, E. M., and Jones, R. T. Long-term survival of normal adult human tissues as xenografts in congenitally athymic nude mice. J. Natl. Cancer Inst. 66: 849–858 (1981).
24. Nettesheim, P., and Griesemer, R. A. Experimental models for studies of respiratory tract carcinogenesis. In: Pathogenesis and Therapy of Lung Cancer (C. C. Harris, Ed.), Marcel Dekker, New York, 1978, pp. 75–188.
25. Nettesheim, P., Klein-Szanto, A. J. P., Marchok, A. C., Steele, V. E., Terzaghi, M., and Topping, P. C. Studies of neoplastic development in respiratory tract epithelium. Arch. Pathol. Lab. Med. 105: 1–10 (1981).
26. Trump, B. F., McDowell, E. M., Glavin, F., Barrett, L. A., Becci, P. J., Schurch, W., Kaiser, H. E., and Harris, C. C. The respiratory epithelium. III. Histogenesis of epidermoid metaplasia and carcinoma in situ in the human. J. Natl. Cancer Inst. 61: 563–575 (1978).
27. McDowell, E. M., McLaughlin, J. S., Merenyi, D. K., Kieffer, R. F., Harris, C. C., and Trump, B. F. The respiratory epithelium. V. Histogenesis of lung carcinomas in the human. J. Natl. Cancer Inst. 61: 587–606 (1978).
28. World Health Organization. Histological Typing of Lung Tumours. 2nd Edition, Geneva, 1981.
29. McDowell, E. M., Wilson, T. S., and Trump, B. F. Atypical endocrine tumors of the lung. Arch. Pathol. Lab. Med. 105: 20–28 (1981).
30. McDowell, E. M., Sorokin, S. P., Hoyt, R. F., and Trump, B. F. An unusual bronchial carcinoid tumor. Light and electron microscopy. Human Pathol. 12: 338–348 (1981).
31. McDowell, E. M., and Trump, B. F. Pulmonary small cell carcinoma showing tripartite differentiation in individual cells. Human Pathol. 12: 286–294 (1981).
32. McDowell, E. M., Harris, C. C., and Trump, B. F. Histogenesis and morphogenesis of bronchogenic neoplasms. In: Morphogenesis of Lung Cancer (Y. Shimosato, M. Melamed, P. Nettesheim, Eds.), CRC Press Inc., Boca Raton, FL, 1982, pp. 1–36.
33. Yuspa, S., and Harris, C. C. Molecular and cellular basis of chemical carcinogenesis. In: Cancer Epidemiology and Prevention (D. Schottenfeld, and J. Fraumeni, Jr., Eds.), W. B. Saunders, Philadelphia, 1982, pp. 23–43.
34. McDowell, E. M., Barrett, L. A., Glavin, F., Harris, C. C., and Trump, B. F. The respiratory epithelium. I. Human bronchus. J. Natl. Cancer Inst. 61: 539–549 (1978).
35. Becci, P. J., McDowell, E. M., and Trump, B. F. The respiratory epithelium. II. Hamster trachea, bronchus and bronchioles. J. Natl. Cancer Inst. 61: 551–561 (1978).
36. Jeffery, P. K., and Reid, L. New observations of rat airway epithelium. A quantitative and electron microscopic study. J. Anat. 120: 295–320 (1975).
37. Spicer, S. S., Mochizuki, I., Setser, M. E., and Martinez, J. R. Complex carbohydrates of rat tracheobronchial surface epithelium visualized ultrastructurally. Am. J. Anat. 158: 93–109 (1980).
38. McDowell, E. M., Barrett, L. A., Harris, C. C., and Trump, B. F. Abnormal cilia in human bronchial epithelium. Arch. Pathol. Lab. Med. 100: 429–436 (1976).
39. Trump, B. F., Saladino, A. J., Berezesky, I. K., and Resau, J. H. A Na ionophore results in metaplasia and hyperplasia in the hamster tracheobronchial epithelium. Fed. Proc. 41: 491 (1982).
40. Sorokin, S. P., and Hoyt, R. F. PAS-lead hematoxylin as a stain for small-granule endocrine cell populations in the lungs and other pharyngeal derivatives and the gut. Anat. Rec. 192: 245–260 (1978).
41. Sorokin, S. P., Hoyt, R. F., and Grant, M. M. Development of neuroepithelial bodies in fetal rabbit lungs. I. Appearance and function maturation as demonstrated by high resolution light microscopy and formaldehyde-induced fluorescence. Exptl. Lung Res. 3: 237–259 (1982).
42. Saccomanno, G., Archer, V. E., Auerbach, O., Saunders, R. P., and Brennan, L. M. Development of carcinoma of the lung as reflected in exfoliated cells. Cancer 33: 256–270 (1974).
43. Schreiber, H., Saccomanno, G., Martin, D. H., Brennan, L. Sequential cytological changes during development of respiratory tract tumors induced in hamsters by benzo[a]pyrene-ferric oxide. Cancer Res. 34: 689–698 (1974).
44. Rous, P., and Kidd, J. G. Conditional neoplasms and subthreshold neoplastic states. A study of the tar tumors of rabbits. J. Exptl. Med. 73: 365–390 (1941).
45. Berenblum, I. The cocarcinogenic action of croton resin. Cancer Res. 1: 44–48 (1954).
46. Mottram, J. C. A developing factor in experimental blastogenesis. J. Pathol. Bact. 56: 181–187 (1944).
47. Boutwell, R. K. The function and mechanism of promoters of carcinogenesis. CRC Crit. Rev. Toxicol. 2: 419–443 (1974).
48. Hecker, E. Cocarcinogenic principles from the seed oil of Croton tiglium and from other euphobraceae. Cancer Res. 28: 2338–2349 (1968).
49. van Duuren, B. L. Tumor-promoting agents in two-stage carcinogenesis. Progr. Exptl. Tumor Res. 11: 31–68 (1969).
50. Slaga, T. J., Sivak, A., and Boutwell, R. K. Carcinogenesis, Vol. 2. Mechanism of Tumor Promotion and Cocarcinogenesis. Raven Press, New York, 1978.
51. Potter, V. R. Initiation and promotion in cancer formation: the importance of studies on intercellular communication. Yale J. Biol. Med. 53: 367–384 (1980).
52. Anonymous. Editorial: The cigarette as co-carcingoen. Lancet i(7220): 85–86 (1962).
53. Roe, F. J. C., Salaman, M. H., and Cohen, J. Incomplete carcinogens in cigarette smoke condensates: tumor promotion by a phenolic fraction. Brit. J. Cancer 13: 623–633 (1959).
54. Auerbach, O., Stout, A. P., Hammond, E. C. and Garfinkel, L. Bronchial epithelium in former smokers. N. Engl. J. Med. 267: 119–125 (1962).
55. Hammond, E. C. Prospects in cancer epidemiology and a theory on carcinogenesis. Environ. Res. 1: 102–113 (1967).
56. Hennings, H., and Boutwell, R. D. Studies on the mechanism of skin tumor promotion. Cancer Res. 30: 312–320 (1970).
57. Trump, B. F., Berezesky, I. K., and Phelps, P. C. Sodium and calcium regulation and the role of the cytoskeleton in the pathogenesis of disease. A review and hypothesis. Scan. Elect. Microsc. 2: 435–454 (1981).
58. Klein-Szanto, A. J., Topping, D. C., Heckman, C. A., and Nettesheim, P. Ultrastructural characteristics of carcinogen-induced dysplastic changes in tracheal epithelium. Am. J. Pathol. 98: 83–100 (1980).
59. Klein-Szanto, A. J., Nettesheim, P., Pine, A., and Martin, D. Distribution and ultrastructural characteristics of dark cells in squamous metaplasias of the respiratory tract epithelium. Am. J. Pathol. 103: 263–270 (1981).